SUB C6 CONT.

B3

polypeptide by the cell [may be] is controlled by altering the concentration of [regulatory drug] tetracycline or an analog thereof to which the cell is exposed [in] after introduction to a mammal.

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17. (Amended) A method of making a physiological composition, the method comprising: obtaining a sample of cells from a mammal; transforming the cells with a nucleic acid sequence encoding a heterologous [immunogenic] polypeptide, said nucleic acid coding sequence being operably linked to a [drug] tetracycline-regulatable promoter; selecting those cells successfully transformed; and mixing the selected cells with a physiologically acceptable diluent.

REMARKS

Claims 1-9 and 11-18 are pending. Claim 10 is canceled. Claims 1, 4-9, 13, 14, and 17 are amended.

At the outset, Applicants wish to thank the Examiner for the telephone interview of September 13, 2000.

The Claimed Invention

The methods of the invention are aimed in general at regulation of the expression of a therapeutic polypeptide in a mammal. The method of the invention involves the administration of cells carrying a repressed, drug-regulatable, therapeutic construct encoding the polypeptide. After administration, the regulatory drug is either administered to the patient or withdrawn, depending upon the exact construct used, in order to de-repress the expression of the therapeutic gene product. One possible advantage of the claimed invention is in that context in which an individual has already raised an immune response to a given polypeptide. In that context, the invention permits the therapy to avoid an immune response to the polypeptide. In this manner, the cells have the opportunity to reach the site at which the product is needed without interference by the immune system. The Examiner's attention is directed to page 8, lines 9-27, which support the summary stated above:

> The invention provides a method of regulating the expression of a nucleic acid sequence encoding a polypeptide which is immunogenic. Preferably, the immunogenic polypeptide is a cytotoxic agent (e.g. an "immunotoxin" - that is, a toxic moiety fused to an immunoglobulin binding domain, or a targeting moiety having a specific binding activity), or an agent such as an immunoglobulin, antibody, bispecific antibody or any of the other known variants of antibodies

(e.g. scFv) capable of recruiting tumour-infiltrating lymphocytes (TILs) into the tumour.

It is preferable to avoid expression of the immunogenic polypeptide until the introduced leukocyte has reached the tumor as it may cause: (a) collateral damage to non-malignant cells and/or (b) interaction of the immunogenic protein with components of the mammal's immune system (especially circulating antibodies). This latter point is particularly pertinent if repeated administrations of the leukocytes is required, as this would cause efficient induction of immune responses to the immunogenic polypeptide, which would tend to interact with the administered leukocytes and prevent them from reaching the target tumour. These problems can be overcome by the method of the present invention, in which the therapeutic (immunogenic) polypeptide is only allowed to become expressed at high levels after a significant time delay, by which point the administered leukocytes will have penetrated the target tumour, thereby preventing interception by the immune system and minimizing collateral damage to non-malignant cells.

Rejection under 35 U.S.C. § 112, first paragraph:

Claims 1-18 are rejected under 35 U.S.C. § 112, first paragraph for overbreadth for a method of regulating the expression of a nucleic acid sequence using a non-tetracycline regulatable system.

Applicants submit that the rejection is obviated by the amendment of the subject claims to specify that the drug-regulatable expression system is a tetracycline-regulatable system. The language "tetracycline or an analog thereof" is found on page 3, lines 13 and 14 of the specification. Applicants respectfully request withdrawal of this §112, first paragraph rejection of claims 1-18.

Claims 11 and 12, drawn to methods of the invention wherein the polypeptide exerts a therapeutic or anti-tumor effect, respectively, are rejected under 35 U.S.C. § 112, first paragraph because the specification allegedly does not provide adequate guidance to obtain any therapeutic effect. First, the Office Action states that Applicants do not teach the route of administration, vector, promoter or dosage that provides efficient gene delivery or the level of protein required to obtain a therapeutic effect. Second, the Office Action argues that given the unpredictability in the art with regard to gene therapy, it would have required undue experimentation to determine the parameters required to obtain a therapeutic effect or anti-tumor effect at the time of filing. Applicants respectfully disagree. These issues are discussed separately below.

Applicants submit that the specification provides guidance with regard to the types of therapeutic gene products useful in the invention, the number of cells to administer that carry the

gene, and the qualities and quantities of a regulatory drug to administer to a patient.[1] Regulation of a transgene in the tissues of a mammal by manipulating the level of a drug had been

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[1] **The specification provides sufficient guidance to enable the invention as claimed:**

The specification provides guidance regarding vectors, promoters, routes of administration, dosages and the level of protein necessary to provide a therapeutic effect. Specific passages providing this information are as follows.

The specification provides guidance on pages 20-21 with regard to the genes of interest or types of antigenic gene products that may be encoded by a cell of the invention and which, when used in the methods of the invention can achieve a therapeutic effect. Examples provided include polypeptides that are antigenic because they are not normally expressed in humans and chimeras of such polypeptides (page 20, lines 27-32), therapeutic proteins that are not expressed in a mammal due to a genetic defect (page 21, lines 5-21) and immunoactive agents (page 21, lines 22-32).

The specification also provides guidance sufficient to enable one of skill in the art to design the vectors necessary for the practice of the invention. An entire section of the specification from page 10 to page 18 is devoted to describing the requirements for the drug-regulatable expression system useful in the invention. Sub-sections describe the critical properties of regulatory promoter systems (page 10, line 20 to page 20, line 14), regulatory promoter systems themselves (page 11, lines 16-27), and details of one such system, the Tet-regulated system. The requirements or characteristics for the vector and expression systems are described both generally and specifically, thereby enabling one of skill in the art to either use the exact system exemplified in detail (the Tet-responsive system) or to apply the characteristics taught to other drug-regulatable vector systems in order to achieve an equivalent effect.

In addition to the section of the specification referred to above, the specification also provides a specific example of the drug-regulatable system and its use in Examples 1 and 2. For instance, Example 1 provides detailed guidance on the generation of a plasmid vector encoding a gene of interest driven by a drug-regulatable promoter, the tTA-responsive promoter. Example 1 also explains that a constitutive promoter may be used to drive the tTA gene. Thus, the specification provides both general and detailed guidance with respect to the vector(s) and promoter(s) useful in the invention.

The specification also teaches at page 16, line 11 that suitable constructs for the Tet regulatable system are disclosed by Hofmann et al. (1996, Proc. Natl. Acad. Sci. U.S.A. 93: 5185-5190; previously made of record, but enclosed for the Examiner's convenience as Exhibit A) and Shockett et al. (1995, Proc. Natl. Acad. Sci. U.S.A. 92: 6522-6526; previously made of record but enclosed for the Examiner's convenience as Exhibit B). Hofmann et al. teaches a retroviral delivery system for tet-regulated genes. The particular vector system described is one in which the withdrawal or absence of tet induces expression of the linked gene of interest. Applicants submit that the reported vector system is directly applicable to the methods of the invention and satisfies the conditions set forth in the specification, and that the specification states as much.

The specification provides guidance with regard to the dosage or number of cells of the invention to be administered to an individual in order to achieve a therapeutic effect on page 20, lines 8-12. This passage also describes acceptable methods of administering cells of the invention to a mammal.

The specification provides guidance with regard to the dosage of regulatory drug to administer in order to achieve a therapeutic effect on page 6, lines 31-32 and on page 20, lines 12-17. In particular, the passage on page 6 states that a dose sufficient "to preferably achieve a serum concentration between approximately 0.05 and 1.0 μg/ml" should be administered. Applicants submit that one of skill in the art may readily determine the amount of a given drug to administer to a mammal to achieve serum concentrations in this range. The key characteristics of a regulatory drug useful according to the invention are discussed on page 18, lines 17-25. Specific characteristics of the tet-regulatory system are described on page 18, line 31 to page 19, line 17. Other drug-regulated expression systems are described on page 19, lines 18-27.

Finally, the specification states at page 18, lines 11-13 that "the desired adjustment of tetracycline concentration in the patient can be made by analysis of clinical signs and symptoms. The additional drug-regulatable promoter systems described above might also be used to regulate the expression of the leukocyte activating molecule". The specification also teaches at page 19, line 28 to page 20, line 6 that:

(continued...)

demonstrated prior to the filing of the present application. The specification teaches monitoring the signs and symptoms of the disease, and provides an example of what constitutes successful application of the invention. Applicants submit that this guidance is adequate for one of skill in the art to achieve and recognize amelioration of a disease or tumor using the methods of the invention.

In the interview of September 13, 2000 it was agreed with the Examiner that the above rejections would be withdrawn if Applicants could document knowledge as of the Applicants' filing date of three elements: a) stable transfection; b) drug regulatable expression of a transgene in vivo; and c) treatment of a tumor via gene therapy. Applicants provide such documentation as follows.

The earliest priority date of the present application is September 6, 1997. The methods of the invention rely upon a novel, unobvious combination of established, predictable methodologies that were well known in the art as of that time. These methodologies include 1) the stable introduction of recombinant constructs to cells in culture, 2) the regulation of a drug-regulated transgene in vivo by changing the concentration of the regulatory drug in vivo, and 3) the treatment of a tumor or other disease by the administration of a recombinant nucleic acid construct encoding a therapeutic polypeptide.

1. Stable transfection

Stable introduction of a recombinant construct to a mammalian cell in culture was known in the art as of that time. Applicants wish to emphasize that the invention entails the introduction of nucleic acid sequences to cells ***in vitro***. The claims do not encompass introducing genes to cells in vivo. Because stable transfection using both plasmids and viral vectors has been a

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(continued...)

> "A therapeutically effective regimen may be sufficient to arrest or otherwise ameliorate the symptoms of a disease. An effective dose regimen requires providing the regulatory drug over a period of time to achieve noticeable therapeutic effects wherein symptoms are reduced to a clinically acceptable standard or ameliorated. The symptoms are specific for the disease in question. For example, when the disease is associated with tumor formation, the claimed invention is successful when tumor growth is arrested, or tumor mass is decreased by at least 50% and preferably 75%."

common laboratory practice for over 20 years, this aspect of the necessary methodologies is fully enabled as of September 6, 1997.

2. Drug-regulatable expression in vivo

The regulation of a recombinant construct by administration of a drug in vivo was established before the earliest priority date of the present application and continues to be borne out by multiple post-filing date publications. For example, Schultze et al. (1996, Nature Biotech. 14: 499-503; referred to in the specification on page 13, line 25 and previously made of record but included here as Exhibit C for the Examiner's convenience.) teaches the "efficient and tight control of reporter gene expression in vitro and in vivo". Schultze et al. demonstrates that up to 800-fold induction of reporter transgene expression can be achieved by manipulating the dose of tetracycline administered to transgenic animals. That is, a drug regulatable promoter system had been demonstrated to function in vivo before the filing of the present application. Further, there is no question that the work of Schultze et al. was scientifically valid, as a multitude of post-filing date publications supports the regulability of drug-regulated promoters in vivo (see, for example, the publication abstracts in Exhibits D-K). Because it is not necessary to describe in detail that which is known in the art in order to provide an enabling disclosure, Applicants submit that the present specification, combined with knowledge available in the art at the time of filing, is fully enabling for the regulation of expression from a drug-regulatable promoter in vivo.

3. Treatment of a tumor or other disease by the administration of a recombinant nucleic acid construct encoding a therapeutic polypeptide

Applicants submit that as of September 6, 1997 there were a number of examples of the use of recombinant vectors encoding a polypeptide for the successful treatment of tumors. For example, Chen et al. (1994, Proc. Natl. Acad. Sci. U.S.A. 91: 3054-3057; Exhibit L) demonstrated that brain tumors (gliomas) in mice were successfully treated using an adenovirus vector expressing herpes simplex virus thymidine kinase (HSV-tk), which rendered tumor cells susceptible to gancyclovir. In this study, 2 out of 10 animals in the experimental group were tumor free and the remaining 8 animals had only small residual gliomas. By comparison, all animals receiving a control vector had tumors that were on average, 23-fold larger than those in the experimental group. This study thus demonstrated successful treatment of mammalian tumors with a recombinant vector.

Addison et al. (1995, Proc. Natl. Acad. Sci. U.S.A. 92: 8522-8526; Exhibit M) demonstrated that intratumoral injection of an adenovirus expressing IL-2 caused tumor regression in a breast cancer model in mice. In one experiment, 8 out of 9 animals showed complete regression of breast tumors after direct injection of virus, and on whole 54% of tumors completely regressed and 33% partially regressed. This study therefore shows the successful treatment of mammalian tumors with a recombinant vector.

Zhang et al. (1996, Proc. Natl. Acad. Sci. U.S.A. 93: 4513-4518; Exhibit N) showed the treatment of several different types of human tumors in a mouse model using an adenoviral vector expressing interferon. In this study, all established breast tumors completely regressed if treatment by viral injection began 15 or 21 days after tumor cell injection. Tumors in 2 of 5 animals in which treatment began 27 days after tumor cell injection showed complete regression. Even the 3 animals in the latter group that did not have complete regression showed partial regression. The Zhang et al. study also examined tumors formed in mice from a human myelogenous leukemia cell line. All such tumors regressed upon injection with the IFN-expressing recombinant viral vector. This study therefore shows the successful treatment of mammalian tumors with a recombinant vector.

Cao et al. (1997 (Feb.), Gastroenterology 112: 501-510; Exhibit O) showed that intratumoral injection of TNF-α-expressing retroviruses was effective to prevent the growth of hepatocellular carcinomas in mice. The intratumoral implantation of TNF-α retrovirus-producing cells completely abolished established hepatocellular carcinoma tumors. This study therefore shows the successful treatment of mammalian tumors with a recombinant vector.

Lukacs et al. (1997 (Apr.), Gene Ther. 4: 346-350; Exhibit P) showed that the introduction of a vector expressing HSP-65 by liposome-mediated gene transfer caused the regression of reticulum cell sarcomas in both immunocompetent and immunodeficient animals. It was noted that the antitumor response in immunocompetent animals was actually greater than in immunodeficient animals. This study shows the successful treatment of mammalian tumors with a recombinant vector.

Block et al. (1997 (Jul.), Pancreas 15: 25-34; Exhibit Q) showed that intrahepatic tumors generated by injection of pancreatic tumor cells could be treated by intratumoral injection of HSV-tk adenoviral particles with intraperitoneal injection of gancyclovir. The intrahepatic tumors are a model of hepatic metastases of pancreatic carcinoma. Highly significant reductions

in tumor volumes were demonstrated using this method, thereby showing successful treatment of a mammalian tumor with a recombinant vector.

Ram et al. (1994, J. Neurosurg. 81: 256-260; Exhibit R) showed treatment of established gliomas in immunocompetent rats with HSV-tk retrovirus and gancyclovir. Tumors were injected with HSV-tk retroviral producer cells and animals were then given gancyclovir injections. The data support the conclusion that tumor eradication involves both gancyclovir killing of virally transduced cells and a disruption of blood supply to the tumor.

In view of the above, Applicants submit that treatment of a tumor using a recombinant vector was well-documented in many prior art references as of the earliest priority date of the application.

Because 1) the stable introduction of recombinant constructs, whether plasmids or viruses, is well known in the art, 2) the ability to regulate a drug-regulatable construct in vivo was described in the specification and was also demonstrated in the art before the earliest priority date of the present application, and 3) the successful treatment of mammalian tumors in vivo using recombinant vectors was not unpredictable as of the earliest priority date of the application, applicants submit that the invention of claims 11 and 12, which recite therapeutic and anti-tumor effects, respectively, is fully enabled. Applicants therefore respectfully request the withdrawal of the §112, first paragraph rejection of claims 11 and 12.

The Office Action also states that the successful treatment of one patient pointed out by Applicants in the discussion of Ross et al. does not make gene therapy predictable. Applicants acknowledge this. However, Applicants were not trying to argue that one patient constituted a predictable method. Rather, Applicants were pointing out that the Ross et al. reference showed success, for example, in shrinking tumor nodules, in a *number* of patients, along with one documented cure. The claims do not require that the claimed invention work in every patient with every recombinant vector, nor does the law require absolute predictability with respect to outcome to meet the enablement requirement.

Applicants' arguments in the previous response with respect to Verma et al. were specifically rejected as non-persuasive in the present Office Action. Applicants argued that the statements in the reference that were relied upon by the examiner to allegedly support the unpredictability of gene therapy did not apply to the presently claimed invention because they were not related to cancer treatment. The present Office Action states that Verma et al.

"correlates with cancer treatment in that it discusses the unpredictability in determining the vector required to obtain a therapeutic level of gene expression using gene therapy." Applicants acknowledge that the reference does make mention of cancer, but submit that it does not relate cancer therapy to the unpredictability of gene therapy. Specifically, the passage on page 241 cited by the examiner states that adenoviral vectors provide a "promising approach" to cancer therapy:

> "One ***promising approach*** is to deliver large numbers of ***adenoviral vectors*** containing genes for enzymes that can activate cell killing, immunomodulatory genes, to cancer cells. In this case, the cellular immune response against the viral proteins will augment tumour killing". (Emphasis added)

Thus, not only does this passage not support the argument that cancer therapy is taught by this reference to be unpredictable, but, because it teaches adenoviral vectors, it does not support the assertion that the reference teaches unpredictability in determining the vector required.

The Office Action also states with respect to Verma et al. that "cancer is specifically mentioned on page 240, Table 1, 4th from the bottom and on page 241, column 2, the end of the second paragraph." Applicants submit that Table 1 on page 240 is titled "Candidate diseases for gene therapy". Applicants submit that this characterization does not support unpredictability of the anti-tumor approach of the invention.

The Office Action states that "Alvarez-Vallina et al. suggests that it was unpredictable what level of expression of a chimeric TCR would be required to obtain T cell activation," and that the specification does not teach the level of expression of a chimeric TCR required to obtain a therapeutic effect or an anti-tumor efffect. Applicants disagree. The specification teaches in detail the amount of chimeric TCR required to **avoid**, as well as to **induce** T cell activation. Specifically, Example 1 teaches in detail how to express a chimeric TCR from an inducible promoter. Example 2 shows that repression of the promoter by the addition of tetracycine reversibly blocks the ability of the cell to be activated in response to antigen. Further, Example 2 demonstrates that responses to antigen are dependent on the dose of regulatory drug present. Applicants respectfully maintain that the teachings of Alvarez-Vallina et al. do not support unpredictability of the present invention. The reference merely states a fundamental of scientific research, i.e., that there is always more to learn. However, even if Alvarez-Vallina et al. is interpreted to indicate unpredictability with respect to the level of chimeric T cell receptor

necessary to obtain T cell activation, the specification supplies adequate guidance in the methods and data presented in Example 2 for one of skill in the art to determine the appropriate level of therapeutic gene expression in leukocytes and how to achieve it.

With respect to regulatory promoter systems, the Office Action states that: "The system used by Applicants provides low gene expression and can be used to prevent toxicity. The claims merely require a regulatable promoter and are not limited to systems that provide low gene expression and can be used to prevent toxicity". Applicants submit that this is a mischaracterization of the invention. While it is true that one aspect of the invention is to maintain the expression of a transgene at a low level for a given interval of time, one must also be able to induce the expression from the transgene in order for the product to have a therapeutic effect. The invention is not functional with low expression alone, but with drug-regulatable expression. Therefore, the Examiner's suggestion that the claims should be limited to a system that provides low expression is improper.

The Office Action states that the specification does not enable regulating the expression of a polypeptide by altering the expression of a regulatory drug after the cell has been administered as encompassed by claim 1 (Examiner's emphasis). Applicants disagree. The specification teaches that the expression of a gene expressed from a drug-regulatable construct may be repressed in vitro by the presence of the drug , (for example, Tet in the system negatively regulated by Tet), and that cells with the drug-regulatable gene in the repressed state are administered to a mammal. In this case, the specification teaches that the "substantial absence" of drug (defined at page 5, lines 7-11) in the mammal induces the expression of the gene of interest. Therefore, Applicants submit that no guidance is necessary regarding a dose of drug to administer to the mammal, since the expression is maximally induced only in the "substantial absence" of the drug.

The specification also teaches that expression repressed by the substantial absence of drug may be "induced after a delay interval by administration to the mammal of the regulatory drug" (page 4, line 32 to page 5, line 6). As noted above, serum concentrations of inducing drug in the range of 0.05 to 1.0 μg/ml are taught at page 6, lines 30-32 and on page 20, lines 16-17. Methods and route of administration of drug are described on page 20, lines 12-14.

Applicants submit, therefore, that the specification fully enables the regulation of a polypeptide by altering the concentration of regulatory drug after the cell has been administered, as encompassed by claim 1 and those claims that depend from it.

The Office Action alleges that claims 4-6 are not enabled since the specification does not teach how to determine whether the mammal has made an immune response, has circulating antibodies, or has immunocompetent memory cells that react with the immunogenic polypeptide as claimed. The Office Action also states that "the specification does not teach how to regulate the expression of an immunogenic polypeptide in" a mammal that has made an immune response to the polypeptide, and that the claims do not provide a nexus between a method of regulating a gene which results in the immune response. The Office Action states that the preamble should reflect the substance of the claim.

Summary of Points Agreed to in the Interview

Applicants wish to summarize the points discussed and agreed to in the telephone interview of September 13, 2000, and to clarify an apparent misunderstanding with regard to the object of the invention. The object of the invention, as summarized above, is to permit the delivery of a therapeutic polypeptide to a site within a mammal without interference by the immune system, particularly when the mammal has already raised an immune response to that polypeptide. The approach has been likened to "Stealth" technology. At the time cells carrying a recombinant nucleic acid construct are administered to a mammal, the expression from the construct is repressed (by either the presence or substantial absence of a regulatory drug), so that the cells can migrate to a desired site of action ("invisible to Radar" so to speak) without being prematurely culled by the immune system. After administration of the cells, the regulatory drug is either initiated or halted, in order to de-repress expression of the polypeptide and effect the therapy. The de-repression takes an interval of time, characterized in the specification as a "delay interval", following administration or removal of the drug. Therefore, in the method of the invention, regulation of the expression of the polypeptide occurs ***both*** in vitro (i.e., repression before administration) and in vivo (i.e., in trans de-repression after administration and change in drug administration), that regulation being dependent on the presence or absence of the drug in both situations.

In view of this characterization of the invention, Applicants emphasize that the object of the invention is ***not*** *to cause or result in an immune response.* Therefore, there need be no

"nexus" between a method of regulating a gene and the immune response to the polypeptide. The limitations to claim 1 introduced in amended claims 4-6 are merely intended to indicate that the method of the invention is functional in an animal that has made an immune response to the polypeptide being expressed from the drug-regulatable promoter before the administration of the cell carrying a construct encoding the polypeptide. As is known in the art, indicators of an immune response to an antigen include the presence of circulating antibodies (for recent antigen exposure) or the presence of immunocompetent memory cells (for less recent antigen exposure) or both. One of skill in the art can readily determine whether an individual has made an immune response to a given polypeptide. Therefore, to the extent that this determination is necessary, claims 4-6 as amended are enabled.

The Office Action states that claims 7-9 are not enabled as written because there is no nexus between the preamble and the body of the claims. The Office Action states that the specification does not enable regulating the expression of a protein in a mammal by regulating the gene while it is within the mammal. Again, this rejection appears to stem from the initial misapprehension of the object of the invention. Applicants submit that the regulation of the expression of a polypeptide in a mammal by altering the concentration of a regulatory drug is enabled as discussed above. That is, the in vivo regulation of a transgene construct by changing the concentration of drug (e.g., Tet) was known in the art and references teaching as much were, in fact, disclosed in the specification (see, for example, p. 13, line 25). In addition, the Office Action states that inhibition of the polypeptide in vitro as recited in claim 7 is not regulating the expression of a polypeptide in a mammal as in the preamble of claim 1. Applicants respectfully disagree with this interpretation of the claims as amended.

The inhibition of expression in vitro is an *additional* step performed, as each of claims 7-9 states, "*prior to introduction of the cell to the mammal...*". The regulation of the expression in a mammal still occurs, as it does in parent claim 1, ***after*** the cell is administered to the mammal. Dependent claims 7-9 function in the same manner and merely emphasize that the baseline expression at the time of administration is low in the case where expression is substantially inhibited in vitro ***before*** the cell is administered to the mammal. The specification teaches substantial inhibition in vitro before administration to a mammal on page 4, line 25 to page 5, line 11.

The Office Action also states that the specification does not enable the term "delay interval" in claim 7. The amendment of claims 7-9 removing this term in favor of the term "2 days" obviates this rejection. Applicants submit that the language "after 2 days" is supported on page 9, lines 17-19.

The Office Action states that the specification does not enable using a viral genome or viral vectors as claimed in claim 13. The Office Action states that given the unpredictability in the art regarding the vector used to obtain the desired result using gene therapy, the vector used is considered essential to the invention. Applicants respectfully disagree. Claim 13 has been amended in order to clarify the language. The Examiner's assertion of non-enablement seems to be based on the alleged unpredictability of the use of viral vectors for gene therapy in vivo. However, the method of amended claim 13 does not require the transduction of cells in vivo with a viral vector. Instead, the method of amended claim 1, from which claim 13 depends, involves the introduction of a cell comprising a vector comprising a nucleic acid encoding a polypeptide wherein the expression of the polypeptide is drug-regulatable. This requires, therefore, that the drug-regulatable nucleic acid sequence be introduced to the cell in vitro, prior to administering the cell to the animal. Therefore, any alleged upredictability stemming from the behavior of viral vectors in vivo would not apply to this claimed method. The cells are transduced with the virus in vitro. As discussed above, the specification provides sufficient guidance, in the form of both specific examples and a description of essential characteristics of the system, for one of skill in the art to design a drug-regulatable expression vector system, including a viral vector system, that will function in the invention. The use of viral vectors to stably introduce gene sequences to cells in culture was well established as of the filing date of the application. Applicants therefore respectfully request that the rejection of amended claim 13 under 35 U.S.C. §112, first paragraph on this ground be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph:

Claims 1 and 7 are rejected under 35 U.S.C. §112, second paragraph as allegedly being unclear for use of the phrase "regulating in a mammal the expression of a recombinant nucleic acid sequence". Specifically, it is said to be "unclear whether applicants intend to regulate the expression in the mammal or in vitro". Applicants submit that, as discussed above, the regulation occurs ***both*** before the cell is administered to a mammal (i.e., repression of expression), and in

the mammal after administration (i.e., de-repression of expression), dependent upon the presence or absence of drug both in vitro and in vivo. Because de-repression cannot occur without prior repression, it is not proper to limit the regulation to one location or the other. Applicants submit that this interpretation is supported in the specification at page 8, lines 9-27. Simply to clarify the claim, Applicants have amended claim 1 to read "A method of regulating [in a mammal] the expression of a recombinant nucleic acid sequence.....so as to achieve in said mammal expression of said nucleic acid sequence, as permitted in the presence or absence of tetracycline or an analog thereof." Applicants submit that this clarification obviates this rejection of amended claims 1 and claim 7 under §112, second paragraph, and respectfully request that the rejection be withdrawn.

Claims 4-6 remain rejected under 35 U.S.C. §112, second paragraph, because of the alleged lack of a nexus between the preamble and substance of the claim. Applicants respectfully emphasize that the body of these claims ***does not result in making an immune response***. Rather, the body of the claim ***results in the drug-regulated expression of a polypeptide in a mammal.*** The body of the claims does not result in inducing circulating antibodies or immunocompetent memory cells. As clearly stated in claims 4-6, the mammal already has made an immune response to the immunogenic polypeptide "prior to said introducing step". The preamble and the body of the claim are therefore fully consistent. Simply in order to clarify that the method is not aimed at raising an immune response, Applicants have deleted the word "immunogenic" from claims 4-6. Applicants submit that these amendments to claims 4-6, are sufficient to overcome this ground of rejection, particularly in view of the discussion herein relating to the object of the invention. Applicants therefore request that this rejection of claims 4-6 as amended be withdrawn.

Claims 7-9 remain rejected under 35 U.S.C. §112, second paragraph for use of the term "delay interval". Applicants submit that this rejection is obviated by amendment of claims 7-9 to recite "after 2 days" instead of "after a delay interval".

Claim 9 remains rejected under 35 U.S.C. §112, second paragraph for use of the term "substantial absence". Applicants submit that the deletion of the word "substantial" in amended claim 9 obviates this rejection.

Claim 14 is rejected under 35 U.S.C. §112, second paragraph as indefinite because it is allegedly unclear what the phrase "in a mammal" refers to, and whether Applicants intend to

claim a cell in a mammal, altering a drug in a mammal or altering expression of a gene in a mammal. Applicants submit that the language "An isolated cell transformed with a nucleic acid sequence" clearly defines the claim as drawn to an isolated cell, not a cell in a mammal, not altering the drug in a mammal, nor controlling the expression of a gene in a mammal. The additional language reciting "such that the expression of the immunogenic polypeptide by the cell is controlled by altering the concentration of regulatory drug to which the cell is exposed" sets forth required characteristics of that isolated cell and the nucleic acid construct is carries. Simply in order to clarify the language of the claim, Applicants have amended claim 14 to recite " altering the concentration of regulatory drug to which the cell is exposed after introduction to [in] a mammal."

Rejection under 35 U.S.C. §102:

Claims 14-17 are rejected under 35 U.S.C. §102 as allegedly being anticipated by Taichman et al. The Examiner states that "The phrase 'may be controlled by altering the... drug to which the cell is exposed in a mammal' is an intended use and may not occur. The phrase does not bear patentable weight when considering the art." The manner of transgene control recited in this phrase was cited by Applicants as clearly distinguishing the invention of claims 14-16 over the disclosure of Taichman et al., which does not teach transgene regulation in a mammal. Claim 14 as amended affirmatively states that "the expression of the immunogenic polypeptide by the cell is controlled by altering the concentration of regulatory drug to which the cell is exposed in said mammal". That is, the amended claim does not recite the regulation of the expression as an intended use that may occur, but as a required characteristic of the cell of the claim. Applicants submit, then, that claim 14 as amended, and therefore claims 15 and 16 which depend from it, are distinguished over the teachings of Taichman et al.

With regard to claim 17, Applicants submit that the culture medium taught by Taichman et al. is not equivalent to the physiologically acceptable diluent specified in the claim and defined in the specification. The definition at page 6, lines 10-12 does not include cell culture medium. Therefore, Taichman et al. does not teach a method involving mixing the selected cells with a physiologically acceptable diluent. Applicants therefore submit that Taichman et al. does not anticipate the invention of amended claim 17 and therefore request that the §102 rejection be withdrawn.

Applicants submit that the above amendments and remarks address all issues raised in the Office Action and respectfully request their entry and consideration.

Respectfully submitted,

10/3/00
Date

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